

In vitro assessments of diverse plant pathogenic fungi treated with a novel growth control agent

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Abstract

The efficacy of an agent with an iodine-based active ingredient (a.i.) was evaluated for controlling the growth of fungi pathogenic to many different food crops. Even though iodine is a necessary mineral for mammals and is an approved food additive, interest in using iodine-based agrochemicals for fungal control is recent. *Fusarium verticillioides* (synonym = *F. moniliforme* J. Sheld) sensitivity to the iodine-base agent was determined using two methods. One method used an agar plug taken from an actively growing culture and the other method used isolated conidia. The concentration of the agent required to inhibit growth of *F. verticillioides* was greater for the culture plug than isolated conidia. Forty-eight strains of *F. verticillioides* were analyzed representing different states within the United States, countries outside the United States, monocot and dicot plant hosts, and mating types. All strains of *F. verticillioides*, regardless of geographic origin, plant host, or mating type were sensitive to the test compound. In addition, 25 species of fungi pathogenic to a diverse array of crops were analyzed and demonstrated to be sensitive to this agent. A pesticide developed with an iodine-based a.i. could be an effective control for diverse fungi pathogenic to a range of plant hosts.

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1. Introduction

In spite of decades of study, one fungal pest that is still without mechanisms for control is *Fusarium verticillioides* (synonym = *F. moniliforme* J. Sheld) (Munkvold and Desjardins, 1997). This fungus has been known nearly a century to be harmful to the growth of maize (Manns and Adams, 1923), one of the world's most important crop plants. However, the dangers posed by this fungus to human and animal health were elucidated only within the last two decades. A series of studies demonstrated that, as this fungus grows on maize kernels, fumonisin toxins are produced. These toxins have been implicated in causing encephalomalacia in horses, edema in pigs, and esophageal cancer in humans (Norred, 1993). Recently, data was obtained that demonstrated fumonisins caused cancer in mice (Howard et al., 2001).

Several investigations have reported potential biocontrol measures for preventing the growth of this organism. Fungus-free maize seedlings were shown to be endophytically infected by a bacterium, *Enterobacter cloacae*, that was isolated and demonstrated to inhibit *F. verticillioides* growth (Hinton and Bacon, 1995). Another fungus, *Trichoderma viride*, also can control growth of *F. verticillioides* conidia (Calistru et al., 1997a,b) and toxin production on maize seed (Yates et al., 1999b). In addition, genetically engineered maize carrying the gene for Bt-toxin reduced *F. verticillioides* infections and toxin production under field conditions (Munkvold et al., 1997, 1998). These studies are promising and would provide alternatives to synthetic pesticides. However, such methods usually require many years of development and testing before becoming commercially available and/or considered acceptable in global consumer markets (Maloy, 1993). Reduced risk pesticides which have little or no mammalian toxicity and are natural in origin could be additional candidates for controlling or suppressing *F. verticillioides* growth.

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Early agriculturalists discovered and used natural substances to their benefit (Koeman and Zadoks, 1999). In two independent studies, an agent with the naturally occurring element, iodine, has been demonstrated to enhance seedling growth following seed treatment to reduce contamination of maize seed with *F. verticillioides* and basil seed with *F. oxysporum* f.sp. *basilicum* (Yates et al., 2003; Adams et al., 2003). Thus, the agent fulfilled the roles of controlling growth of the fungal pest while enhancing growth of the plant host. However, the agent reduced yields of strawberry plants when used as a soil amendment to replace methyl bromide for controlling nematodes, weeds, and soilborne pathogens (Kokalis-Burelle, 2003). Furthermore, iodine has been identified as an essential mineral in the human diet with 150 µg as the recommended dietary allowance for adults (Miller, 1996). The dose–response relationship between mineral intake and physiological function is a bell-shaped curve demonstrating an optimum in the center with both excessively low and high intakes resulting in deficiencies and toxicities, respectively (Miller, 1996). The objective of the current research was to determine whether this iodine-based agent could control the growth of *F. verticillioides* isolates originating from different geographic locations, host plants, and mating types. In addition, the efficacy of the agent was analyzed for a diverse array of fungal genera pathogenic to many different crop plants.

2. Materials and methods

2.1. Source of fungal specimens

F. verticillioides cultures were purchased from the *Fusarium* collection maintained at Kansas State University (John Leslie, Manhattan, KS) with the exception of a genetically transformed strain designated RRC PATg (Yates et al., 1999a). The RRC PATg was developed from a wild-type strain designated RRC PAT of mating population A that was isolated from maize grown in the southeastern United States (Bacon and Hinton, 1996). These two latter cultures have been used extensively in our lab for other studies and PATg was used as the control in each assay. The PATg strain contained the *gusA* reporter gene which encodes β-glucuronidase (GUS) and the *hph* gene for hygromycin resistance as the selectable marker (Yates et al., 1999a). PATg has been demonstrated to mirror PATwt for all characteristics examined to date including (1) growth rate on various media (Yates et al., 1999a); (2) level of production of the mycotoxin, fumonisin B₁ (Yates et al., 1999b); (3) inhibition of growth and mycotoxin production by the biocontrol fungus, *T. viride* (Yates et al., 1999b); (4) mycelial proliferation dependence on maize tissue type, maturity, and physical condition (Yates and

Jaworski, 2000); and (5) conidial growth inhibition at <10 µg/ml and slight enhancement at <1.0 µg by an iodine-containing agent, AJ1629-34EC, formerly identified as Plantpro (Yates et al., 2003). Therefore, the PATg transformant was used in the current studies.

The *F. graminearum* sample was a composite mixture of strains from maize (28718, 29020, 29105, 5883) and wheat (28063, 28336) obtained from the Agriculture Research Service Culture Collection (O'Donnell et al., 2000). All other species of pathogenic fungi were obtained from the American Type Culture Collection (Manassa, VA).

Primary cultures of each fungal specimen were propagated on solidified medium recommended by the supplier. Conidia were collected from actively growing cultures and stored at 5°C on silica gel (Perkins, 1962) until used for experiments. Cultures were prepared from the silica gel stored samples for analyses of the test agent.

2.2. Assay of fungal sensitivity to the test agent

Isolated conidia of *F. verticillioides* specimens were prepared for analyses from cultures grown on potato dextrose agar (PDA) (Becton Dickinson Microbiology Systems, Sparks, MD). The one exception was the transformed strain, *F. verticillioides* RRC PATg, which was genetically engineered for hygromycin resistance. This strain was propagated on minimal medium (Puhalla and Spieth, 1983) containing 100 µg/ml hygromycin B (MMH) (Boehringer Mannheim Corp., Indianapolis, IN). Cultures were incubated for 7 days at 25°C to 27°C on a 12 h light/dark cycle. Conidia were collected for experimentation by flooding the agar surface of a culture with 10 ml of sterile distilled water (SDW). A hemacytometer (Hausser Scientific Co., Horsham, PA) was used to count conidia for determining the concentration of conidial suspensions.

F. verticillioides PATg conidia at 2.0×10^4 conidia/ml were assayed for sensitivity to the active ingredient in AJ1629-34EC (Ajay North America, L.L.C., Powder Springs, GA). Concentrations of AJ1629-34EC active ingredient (a.i.) examined were 0, 1.0, 5.0, 10.0, 25.0, and 50.0 µg/ml as determined by standard analytical techniques (American Chemical Society, 1993; Furman, 1962). Immediately after suspending conidia in the diluted AJ1629-34EC solution, 10 µl of conidial suspension was spread onto PDA agar in a 100 mm × 25 mm petri dish and incubated in the dark at 25°C. The number of visible colonies was recorded after 4 days incubation (Fig. 1A).

A second method was used to test the efficacy of AJ1629-34EC for controlling fungal growth by removing a 4 mm² agar plug from cultures actively growing on solidified medium. The sensitivity of *F. verticillioides* RRC PATg, as well as many other genera of fungal

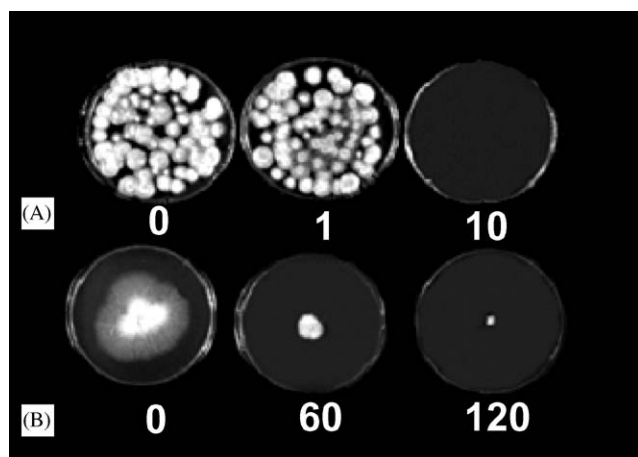


Fig. 1. *Fusarium verticillioides* RRC PATg growth 4 days after treatment. (A) Isolated conidia suspended in 0, 1, and 10 µg/ml of AJ1629-34EC active ingredient (left to right) and then immediately removed and spread on solidified potato dextrose agar contained in petri dishes. (B) A 4 mm² plug was removed from an actively growing culture and the growing surface placed in contact with solidified agar on which 0, 60, and 120 µg/ml of AJ1629-34EC active ingredient (left to right) had been spread and dried for 1 h.

plant pathogens, were analyzed by this method. The medium for growth from the mycelial plug was solidified 4.9% PDA. The test agent was added to the surface of solidified agar to avoid the possibility of any chemical alterations occurring to the test agent caused by heat generated in molten agar. A 4.9% PDA was used, instead of the standard 3.9%, to facilitate diffusion of AJ1629-34EC into the solidified medium. A 1 ml sample of each concentration of AJ1629-34EC a.i. (0, 3, 30, 60, 120, 180, 240, and 300 µg/ml) was spread onto the corresponding designated plate and 1 ml of sterile, distilled water, onto designated control plates. Plates were air-dried overnight in a laminar flow hood until no liquid was visible on the agar surface. Each plate was inoculated in the center by placing the growing surface of a 4 mm² plug cut from a 7- to 14-day-old culture of each fungal strain adjacent to the agar surface. Cultures were grown in the dark at 25°C in an incubator (Harris Manufacturing Co., Asheville, NC). The diameter of the resultant growth was measured after four days (Fig. 1B).

2.3. Data analysis

Experiments for each fungus consisted of six replications of each AJ1629-34EC a.i. concentration and were repeated three times. For assays using isolated *F. verticillioides* conidia, the percentage of colonies surviving at a given concentration was calculated relative to the number of colonies without AJ1629-34EC set at 100% survival. For assays using agar plugs, the plug diameter at a given concentration for cultures treated

with AJ1629-34EC was calculated relative to the growth without AJ1629-34EC set at 100% survival.

Survival data for each fungal isolate obtained by analyses of either isolated conidia or culture plug were treated to curve fitting analyses using the equation $\ln y = a + bx$ (TableCurve, Jandel Scientific, San Rafael, CA). The EC₅₀ was derived from the curve as the concentration of AJ1629-34EC resulting in a 50% survival. In addition to treating data of each fungal isolate individually to curve fitting analyses, the combined data for all fungi obtained from the ATCC was treated by curve fitting to develop an expression of the sensitivity of pathogenic fungi in general to this agent.

2.4. Microscopy

A microscopic comparison was made between samples prepared for analysis by isolated conidia and by 4 mm² culture plugs of *F. verticillioides* PATg. Conidia were collected by flooding the surface of a culture with sterile distilled water. Samples were prepared from a culture plug by suspending a 4 mm² plug in 1 ml of water in a sterile tube. The tube was agitated using a vortex mixer at low speed for 2 min. Each of the resultant suspensions was mounted on a slide for examination with a microscope (model 22 EB, Leitz Dialux; LEICA, Wetzlar, Germany).

3. Results and discussion

3.1. *F. verticillioides* sensitivity to the test agent compared in two methods of *in vitro* analyses

Assessment values differed between isolated conidia and culture plugs for the concentration of AJ1629-34EC at which *F. verticillioides* RRC PATg growth was inhibited by 50% (EC₅₀) (Tables 1 and 2). The EC₅₀ was lower for isolated conidia at 2×10^4 conidia/ml than for a 4 mm² culture plug. The EC₅₀ was 3.2 µg/ml AJ1629-34EC for isolated conidia and 55.3 µg/ml for a 4 mm² culture plug. Differences in the methodology of the two assays may account for the results. One difference in the methodologies is the cellular structure treated in each assay. Only conidia are present in the isolated conidia method (Fig. 2A), but both conidia and mycelia are present in the culture plug method (Fig. 2B). Perhaps, conidia are more sensitive than mycelia to AJ1629-34EC which results in a lower concentration to generate an EC₅₀ for conidia. A second difference is the physical state of the medium with which fungal structures are in contact during exposure to the test agent. Conidia are suspended in a liquid dilution of the agent for about 1 min before being spread on solidified agar; whereas, in the culture plug method, the agent is

Table 1

Sensitivity of *F. verticillioides* strains from throughout the United States to an iodine-based agent

Location	Collection#	Host	Mating type	EC50 ^a
Alabama	A-2925	Peanut	A +	2.9 (2)
Alabama	A-2976	Soil	A +	4.3 (2)
Arkansas	A-1221	Sorghum	A +	2.9 (2)
California	A-0091	Maize	A +	7.8 (3)
Georgia	A-1041	Maize	A +	3.9 (1)
Georgia	RRC PATwt	Maize	A +	3.2 (3)
Georgia	RRC PATg	Maize	A +	3.2 (3)
Illinois	D-1007	Grass	D +	2.6 (2)
Kansas	A-2182	Maize	A +	4.3 (3)
Kansas	A-0503	Maize	A-	3.5 (1)
Kansas	D-0479	Maize	D +	3.4 (2)
Kansas	D-0550	Maize	D-	3.6 (2)
Kansas	E-0731	Maize	E +	3.3 (4)
Kansas	F-1137	Sorghum	F +	3.6 (4)
Kansas	F-1054	Sorghum	F-	2.9 (1)
Kansas	D-0741	Shattercane	D +	1.7 (4)
Missouri	D-1026	Grass	D +	2.7 (4)
North Carolina	A-1012	Maize	A +	7.9 (4)
South Carolina	A-1017	Maize	A +	5.4 (2)
Texas	F-4084	Peanut	F +	2.1 (2)

^a EC50 = Concentration of AJ1629-34EC active ingredient (µg/ml) at which survival was 50% as determined by treating data to curve fitting analyses. The number in parenthesis indicates the curve from which EC50 was evaluated: (1) $\ln y = a + bx$, (2) $\ln y = a + bx^2$, (3) $\ln y = a + bx^3$, (4) $y^{-1} = a + bx^3$. The r^2 for all curves was ≥ 0.90 .

diffused into solidified medium on which the plug is placed. A third difference is the number of fungal structures exposed to the test agent. Microscopic examination demonstrated the incidence of fungal structures in an isolated conidia sample diluted to 2×10^4 conidia (Fig. 2A) is lower than in a sample washed from a culture plug (Fig 2B). In assays with isolated conidia, a suspension containing about 200 conidia was spread across the agar surface. In contrast, a water wash of a 4 mm² culture plug was determined to contain approximately 7.4×10^7 conidia as determined by counts made from samples mounted on a hemacytometer.

The logic for treating at a cellular concentration of 2×10^4 conidia/ml was based on the method used for the evaluation of isolated conidia. The number of colonies visible on a 100 mm \times 15 mm petri dish was counted after 4 days growth from a 10 µl conidial suspension spread on the plate. Control cultures without any exposure to the test agent would be expected to produce about 200 colonies/plate, the maximum number easily differentiated into discrete colonies after four days growth. An advantage to using isolated conidia for analyses is that the biological load, that is, the type and numbers of fungal structures, can be quantified and adjusted to a consistent value among experiments and *F. verticillioides* strains. A major disadvantage of isolated

Table 2

Sensitivity to an iodine-based agent for *F. verticillioides* strains from different countries

Location	Collection #	Host	Mating type	EC50 ^a
Argentina	A-4856	Maize	A +	11.6 (4)
Brazil	A-0206	Sorghum	A +	1.4 (4)
Brazil	A-0207	Sorghum	A-	3.1 (3)
China	A-1562	Maize	A +	6.8 (3)
Costa Rica	A-7237	Maize	A +	5.9 (1)
Egypt	A-4362	Sorghum	A +	3.3 (2)
Egypt	A-4367	Maize	A-	13.7 (2)
Egypt	D-4375	Cotton	D +	2.5 (4)
Egypt	F-4372	Sorghum	F +	1.2 (2)
Egypt	F-4370	Sorghum	F-	0.92 (3)
Germany	A-3824	Maize	A-	6.0 (1)
Germany	B-3828	Cattleya	B +	5.3 (4)
Italy	A-0171	Rice	A +	2.3 (4)
Kenya	A-5461	Maize	A +	2.8 (4)
Malaysia	A-6054	Maize	A +	8.2 (4)
Mexico	A-0354	Maize	A +	1.8 (4)
Nepal	A-4516	Maize	A +	13.2 (4)
Nigeria	A-4934	Millet	A +	13.5 (4)
Phillippines	B-1728	Sorghum	B +	22.6 (4)
South Africa	A-4801	Maize	A +	3.0 (4)
South Africa	F-3884	Sorghum	F +	3.1 (4)
South Africa	F-3869	Sorghum	F-	3.6 (4)
Taiwan	A-0273	Sugarcane	A-	3.4 (4)
Thailand	A-3957	Maize	A +	5.1 (4)
Thailand	A-4426	Banana	A +	5.9 (4)
Turkey	A-3823	Banana	A-	2.1 (1)
Uruguay	A-8269	Maize	A +	8.8 (4)
Venezuela	A-0218	Maize	A-	2.1 (4)

^a EC50 = Concentration of AJ1629-34EC active ingredient (µg/ml) at which survival was 50% as determined by treating data to curve fitting analyses. The number in parenthesis indicates the curve from which EC50 was evaluated: (1) $\ln y = a + bx$, (2) $\ln y = a + bx^2$, (3) $\ln y = a + bx^3$, (4) $y^{-1} = a + bx^3$. The r^2 for all curves was ≥ 0.90 .

conidia is the increased time required for sample preparation for determining and adjusting conidia concentration. In contrast, an advantage of the culture plug method is that the time required for sample preparation is minimal. Consequently, the number of strains that can be assayed within a given time is maximized thereby reducing unknown variables that could occur over longer periods of time required for sample preparation. However, a major disadvantage of the culture plug method is that the biological load can not be accurately quantified.

3.2. Analyses of *F. verticillioides* from diverse geographic locations, plant hosts and mating types

The taxonomic nomenclature for *Fusarium* sp. has been in a state of flux for several years (Leslie, 1995). The morphologically similar strains once classified as *F. moniliforme* or *verticillioides* have been differentiated into other species based on reproductive isolation and/

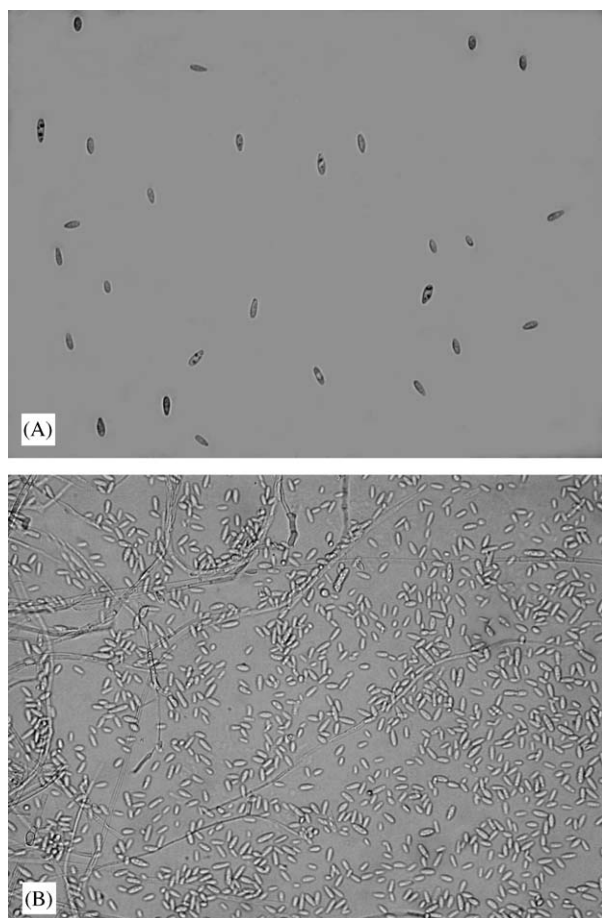


Fig. 2. Comparison between *F. verticillioides* PATg samples prepared for analysis by the isolated conidia and 4 mm² culture plug methods. (A) Isolated conidia at 2×10^4 conidia/ml as used in the assays. (B) Suspension from culture plug following agitation in 1.0 ml of water and mounted on a hemacytometer for examination with a microscope.

or molecular characterization. In the current study, the morphological convention for nomenclature has been used by referring to all strains as *F. verticillioides*. However, each strain has been identified by their Kansas State University collection numbers in Tables 1 and 2 should clarification be required on species nomenclature based on other criteria.

Previous research had demonstrated sensitivity to AJ1629-34EC for an *F. verticillioides* wild type isolated from maize growing in the southeastern United States and a genetically transformed isolate developed from the wild type. However, the possibility exists that geographic isolation could result in ecotypic variants that would not respond to the test agent, AJ1629-34EC. *F. verticillioides* is considered sensitive to hygromycin B₁ (Leslie, 1995); however, resistant strains have been isolated from corn fields in middle Georgia (Yates et al., unpublished data). In order to determine the influence of geographic origin, 47 additional *Fusarium*

sp. strains were tested representing ten states within the United States (Table 1) and nineteen countries outside the United States (Table 2).

F. verticillioides growth was controlled by an agent with an iodine-based a.i. regardless of the geographic origin of the strain within the United States (Table 1). The location of the states varied from as far east as the Atlantic coast, Georgia and North Carolina, to as far west as the Pacific coast, California. Likewise, growth was controlled for strains originating from southern and northern states as represented by Texas and Illinois, respectively. Not only was AJ1629-34EC effective in controlling growth of *F. verticillioides* strains originating from the United States, but also from outside the United States. The continents from which strains originated were from North America, South America, Africa, Europe and Asia.

Adaptations made by a fungus in order to grow on a particular host plant or substrate possibly could be another factor influencing fungal sensitivity to a test agent. Consequently, *F. verticillioides* strains isolated from various host plants were analyzed for sensitivity to AJ1629-34EC. Maize was the host plant for the majority of the *F. verticillioides* strains analyzed (Tables 1 and 2). All cultures originated from plants within the Angiosperm phylum with the majority isolated from monocot hosts. The monocot hosts in addition to maize were sorghum, shattercane, cattleya, rice, sugarcane, and banana. Dicot hosts included cotton and peanut. AJ1629-34EC effectively controlled the growth of *F. verticillioides* originating from all these host plants. Likewise, mating type of the strain did not generate resistance to AJ1629-34EC. The most common mating type analyzed was A+. Other mating types analyzed were A–, B+, D+, D–, E+, F+, and F–. Growth of all mating types was effectively controlled by the test agent.

3.3. Analyses of diverse genera of pathogenic fungi

A variety of genera of plant pathogenic fungi demonstrated sensitivity to AJ1629-34EC analyzed by the plug method (Table 3). The genera assayed included *Alternaria*, *Aspergillus*, *Cercospora*, *Colletotrichum*, *Didymella*, *Elsinoe*, *Glomerella*, *Mycosphaella*, *Phomopsis*, *Phytophthora*, *Pyrenophora*, and *Septoria*. In addition, *F. graminearum* and *F. proliferatum* were analyzed. The host plants for these fungal pathogens included citrus, cucumber, grape, peanuts, maize, wheat, potato, sugar beets, tomatoes, and turf grass. The EC₅₀ was 30 µg/ml for the majority of the fungal genera.

A survival curve was developed for the response of plant pathogenic fungi to AJ1629-34EC (Fig. 3). The data analyzed consisted of the percent survival of the 22 different fungal species treated to AJ1629-34EC a.i. at concentrations of 3, 30, 60, 120, 180, 240, and 300 µg/ml.

Table 3
Sensitivity to an iodine-based agent of diverse plant pathogenic fungi genera

Fungus	Host	ATCC ^a	EC50 ^b
<i>Fusarium verticillioides</i> PATg	Maize		55.3 (1)
<i>Elsinoe fawcettii</i> Jenkins	<i>Citrus tangerina</i>	13200	7.7 (1)
<i>Alternaria citri</i> Ellis et Pierce	Citrus fruit	24463	31.4 (1)
<i>Colletotrichum gloeosporioides</i> Penzig	Grapefruit	36036	26.9 (1)
<i>Mycosphaerella citri</i> Whiteside	Grapefruit leaf	24046	45.4 (1)
<i>Alternaria cucumerina</i> (Ellis et Everhart) Elliott	Cucumis melo leaf	60256	57.7 (1)
<i>Glomerella cingulata</i> var. <i>orbiculare</i> Jenkins et Windstead ^c	Cucumber	11326	35.9 (1)
<i>Didymella bryoniae</i> (Auerwald) Rehm	<i>Cucumis sativus</i>	200532	113.3 (3)
<i>Phomopsis viticola</i> (Saccardo)	Grapevine wood	76192	4.2 (3)
<i>Cercospora arachidicola</i> Hori	<i>Arachis hypogaea</i>	18667	5.2 (3)
<i>Fusarium graminearum</i> ^d	Maize and wheat ^d	See ^d	23.2 (1)
<i>Colletotrichum graminicola</i> (Cesati) Wilson	<i>Zea mays</i>	34167	60.9 (1)
<i>Fusarium graminearum</i> Schwabe	Corn stalk	15624	28.0 (1)
<i>Aspergillus flavus</i> Link	Peanuts	24131	40.4 (1)
<i>Colletotrichum graminicola</i> (Cesati) Wilson ^c	Peanut plant	18415	31.2 (3)
<i>Alternaria solani</i> (Ellis et Amartin) Sorauer	Potato	38918	27.4 (3)
<i>Pyrenophora teres</i> Drechsler f. <i>teres</i>	<i>Hordeum vulgare</i>	52700	6.9 (1)
<i>Septoria avenae</i> f. sp. <i>triticea</i> Johnson	<i>Hordeum jubatum</i>	26374	44.4 (1)
<i>Fusarium proliferatum</i> (Matsushima) Nirenberg ^f	<i>Oryza sativa</i>	12617	29.4 (1)
<i>Cercospora beticola</i> Saccardo	Sugar beet leaves	18368	43.9 (1)
<i>Colletotrichum atramentarium</i> (Berkeley et Broome) Taubenhau ^g	<i>Lycopersicon esculentum</i>	76325	22.8 (1)
<i>Phytophthora infestans</i> (Montagne) de Bary	<i>Lycopersicon esculentum</i>	48718	64.6 (1)
<i>Fusarium oxysporum</i> Schlechtendahl	Turf grass roots	12581	50.1 (1)
<i>Saccharomyces cerevisiae</i> Hansen	Distillery yeast	9763	133 (2)

^aNomenclature = host and pathogen names, authors, and numbers are as identified by the American Type Culture Collection (ATCC), with the exception of *F. verticillioides* PATg and the composite *F. graminearum* culture which are described in materials and methods.

^bEC50 = Concentration of AJ1629-34EC active ingredient ($\mu\text{g/ml}$) at which survival was 50% as determined by treating data to curve fitting analyses. The number in parenthesis indicates the curve from which EC50 was evaluated: (1) $\ln y = a + bx$, (2) $\ln y = a + bx^3$, (3) $\ln y = a + bx^{0.5}$. The r^2 for all curves was ≥ 0.90 .

^cDeposited as *Colletotrichum lagenarium*.

^dComposite sample from the Agriculture Research Service Culture Collection composed of a mixture of strains from maize (28718, 29020, 29105, 5883) and wheat (28063, 28336).

^eDeposited as *Volutella* sp.?

^fDeposited as *Gibberella fujikuroi*.

^gDeposited as *Colletotrichum coccodes* (Wallroth) Hughes, anamorph.

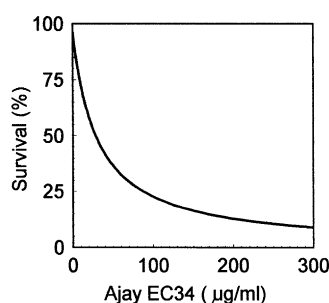


Fig. 3. A survival curve based on the response of 22 different plant pathogenic fungi to AJ1629-34EC active ingredient at 0, 3, 30, 60, 120, 180, 240, and 300 $\mu\text{g/ml}$. The data set was treated to Lowess data smoothing (40%) and determined to fit to the following curve: $1/y = a + bx$; Fit Std Err = 4.308; Fstat = 12833 where $a = 0.010398177$, $b = 0.00033944042$; r^2 coef det = 0.98.

The curve can be used to extrapolate the percent reduction in survival expected with the application of any given amount of AJ1629-34EC over a range of fungal species similar to those treated in the current

study. For example, a reduction in survival of 20%, 40%, 60%, and 80% can be expected with 5, 20, 40, and 120 μg AJ1629-34EC/ml.

Our interest in determining the potential of AJ1629-34EC as a fungicide was generated because the active ingredient was based on a food additive, iodine. Iodine is a naturally occurring element commonly found in nature (Chilean Iodine Educational Bureau, 1951). Iodine-containing products are not toxic to humans at appropriate concentrations and small amounts of iodine are required for proper functioning of the thyroid glands (Miller, 1996). Furthermore, iodine-based materials have been used as microbial control agents in human and animal health for centuries (Nebergall and Schmidt, 1957). Consequently, obtaining approval for commercial use of an iodine-based, reduced risk pesticide may prove less complex, time consuming, and costly than for a typical synthetic pesticide or an introduced micro-organism. Iodine-based materials have not been commonly used as fungicides. The lack of interest in using iodine-based agents as a fungicide may relate to plant

nutritional studies in the first part of the 20th century reporting that iodine-containing materials could be toxic to plants (Cotton, 1930; Wynd, 1934). However, our previous research demonstrated enhanced shoot and root development for plants grown from *F. verticillioides*-inoculated maize seed treated with 10 mg a.i. AJ1629-34EC/kg seed (Yates et al., 2003).

The iodine-based a.i. of AJ1629-34EC functioned to control *F. verticillioides* collected from diverse geographic locations throughout the world, plant hosts of both monocot and dicot species, and different mating types. Not only is this material effective in controlling *F. verticillioides* from diverse geographic locations and hosts, but also other species of *Fusarium* and other fungal genera pathogenic to many important crops.

3.4. Summary

The current study demonstrated growth control of *F. verticillioides* RRC PATg by AJ1629-34EC, an iodine-based agent, using two methods of in vitro analyses, isolated conidia and culture plug. The advantage of using isolated conidia is that the biological load can be determined which is not possible with a culture plug. The advantage of using a culture core is that sample preparation time is minimal, whereas, isolated conidia require a much longer time. The agent controlled growth of *F. verticillioides* originating from many different geographic sites and host plants, as well as a diverse array of other genera of plant pathogenic fungi. These results indicate the agent may have a broad spectrum of fungicidal activity. Additional research is warranted to determine the performance and yield of greenhouse and field grown plants following treatment. Theoretically, control with AJ1629-34EC may prove less detrimental to the ecosystem than other types of controls because the active ingredient is a naturally occurring element.

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